2G1424 蛋白質毒素ライセニンがスフィンゴミエリンを含む脂質膜中に誘起するポア形成：単一 GUV 法による研究**Single Giant Unilamellar Vesicle Method Reveals Lysenin-Induced Pore Formation in Lipid Membranes Containing Sphingomyelin**

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Lysenin is a sphingomyelin (SM)-binding pore-forming toxin. To reveal the interaction of lysenin with lipid membranes, we investigated lysenin-induced membrane permeation of a fluorescent probe, calcein, through dioleoylphosphatidylcholine (DOPC)/SM, DOPC/SM/cholesterol (chol), and SM/chol membranes, using the single giant unilamellar vesicle (GUV) method¹. The membrane permeability coefficient of calcein increased over time to reach a maximum, steady value, P^s , which persisted for a long time (100-500 s), indicating that pore concentration increases over time and finally reaches its steady value. The P^s values increased as the SM/lysenin ratio decreased, and at low concentrations of lysenin the P^s values of SM/DOPC/chol (42/30/28)-GUVs were much larger than those of SM/DOPC (58/42)-GUVs. The dependence of P^s on the SM/lysenin ratio for these membranes was almost the same as that of the fraction of sodium dodecyl sulphate (SDS)-resistant lysenin oligomers. On the other hand, lysenin formed pores in GUVs of SM/chol (60/40) membrane in homogeneous liquid-ordered phase, indicating that the phase boundary is not necessary for the pore formation. The P^s values of SM/chol (60/40)-GUVs were smaller than those of SM/DOPC/chol (42/30/28)-GUVs even though the SDS-resistant oligomer fractions was similar for both the membranes, suggesting that there are irreversible oligomers (or pre-pores) before conversion to pores. Based on these results, we discuss the elementary processes of lysenin-induced pore formation.

1. *Biochemistry, in press*

2G1436 人工細胞ベシクルにおける脂質ドメインへの化合物局在を介したドメインサイズと膜崩壊率の制御**Localization of bulky-molecules in raft-like domains on phase-separated liposomes: Control of domain size and bursting rate of liposome**

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It is established that the cell-sized liposomes composed of a saturated lipid DPPC, an unsaturated lipid DOPC, and cholesterol (Chol) show phase separation below the transition temperature, and form domain structures like lipids rafts [1]. These micro-domains grow up to mono-domain, so as to minimize interfacial instability. By applying the phase separation of lipids, we report two control methods, i.e., a size-control of domains by the addition of poly(ethylene glycol)-conjugated Chol (PEG-Chol) [2], and a rate-control of UV-induced bursting of liposomes by the addition of Azo-TAB molecules [3]. As for the domain-size control, dispersed micro-domains <1μm were observed in the presence of PEG-Chol above a critical composition. The transition from global- to micro-segregation is interpreted in terms of the competition between steric repulsive interaction between the bulky-head groups of PEG-Chol and the cost in line energy along the domain boundaries. On the other hand, the addition of Azo-TAB into the membrane expands it and induces bursting of liposomes due to the UV irradiation. Furthermore, the bursting rate was controlled by the area fraction of domains rich in DPPC. These achievements lead us to further understanding the lipid raft structure from physicochemical view.

[1] M. Yanagisawa et al., 2007 Biophys. J. 92:115.

[2] M. Yanagisawa, et al., 2012 Soft matter, 8:488.

[3] A. Diguët, et al., 2012 J. Am. Chem. Soc., 134:4898.

2G1448 PI4P と脂質膜曲率による Kes1 ステロール輸送活性の制御**Regulation of Kes1 sterol transport activity by PI4P and lipid membrane curvature**

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Kes1 is one of seven members of the yeast Oxysterol binding protein homolog (Osh) family. This protein transfers sterols between plasma membrane and the Golgi, and regulates Golgi secretory function. However, these detailed mechanisms have not been well understood. In this work, we investigated the effect of lipid membrane state (lipid composition and membrane curvature) on Kes1 activities (sterol extraction and transport) by fluorescence resonance energy transfer. Especially we focused on phosphatidylinositol 4-phosphate (PI4P) because PI4P is considered to stimulate Kes1 sterol transport activity. Effects of membrane curvature were evaluated by using liposomes with different size. For planar membranes, while two anionic lipids, phosphatidylinositol and phosphatidylserine, had little effect on the Kes1 activities, PI4P was found to affect on the Kes1 activities depending on PI4P amount: Although a small amount (1 mol%) of PI4P stimulated sterol extraction and transport by Kes1, larger amount (~10 mol%) of PI4P inhibited the Kes1 activities. This sterol extraction inhibitory effect of PI4P was dependent on the membrane curvature. That is, even 1 mol% of PI4P inhibited the sterol extraction from highly curved membranes, suggesting that the membrane curvature regulates the effect of PI4P. These findings suggest a possibility that PI4P and the membrane curvature are regulating factors of Kes1 biological function.

2G1510 リン脂質輸送タンパク質 Sec14 の脂質輸送メカニズムの解明**Elucidation of lipid transfer mechanism of phospholipid transfer protein Sec14**

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Sec14, a major yeast phosphatidylinositol/phosphatidylcholine transfer protein (PITP) regulates lipid membrane trafficking from the trans-Golgi network. However, its detailed mechanism remains to be elucidated. In this work, we investigated PITP-membrane interactions by surface plasmon resonance (SPR) and fluorescence spectroscopy. Sec14 WT and two mutants, which have been designed to ablate their phosphatidylcholine (PC)-binding ability (Sec14 II) and phosphatidylinositol (PI)-binding ability (Sec14 AD) were used and compared. In the fluorescence spectroscopy, capture of pyrene-labelled PC by PITPs from large unilamellar vesicles (LUVs) was detected by the decrease in the pyrene excimer/monomer intensity ratio. The results showed that Sec14 WT and its mutants, including PC-binding mutant (Sec14 II), have PC transfer activity. In the presence of PI, however, PC transfer activity for Sec14 II was reduced, suggesting that Sec14 II has higher affinity for PI.

Binding behavior of PITPs to immobilized LUVs was evaluated by SPR method. Sec14 II exhibited higher binding to PC membrane than Sec14 WT and AD, which have intact PC binding pocket. On the other hand, membrane binding of Sec14 II was decreased in the presence of PI, Ligand for this mutant. These findings that Sec14 represents reduced affinity to the membranes that contain ligands for the protein suggest the ingenious lipid transfer mechanism, by which Sec14 dissociates from the membrane when it captures PC or PI in appropriate position of the binding pocket.

2G1522 ゲル、アミノ酸、水素化アモルファスシリコンを用いた光制御イオン伝導整流素子**Photo-controlled ion conductive rectification element using gel, amino acids and hydrogenated amorphous silicon film**

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Ionic conduction, in solution as well as in intermediate states between a liquid and a solid, such as gels, is a phenomenon key to inter-conversion between light energy, chemical energy, and electric energy. As such, this phenomenon is essential for devices that store, generate, and convert electric energy, including batteries, photovoltaics, capacitors, and actuators. While not dismissing these applications, this article focuses on ionic conduction in nontoxic materials to discuss the creation of elements coated with multi-layer functional thin-films using a combination of different technologies, such as the rectification property of ionic conduction, photo-induction control, and the use of wavelength conversion materials, and the potential application of these materials to biological tissue. Amino acid is the elementally element of bio molecule, has potential of diversity to electro chemical device. Our group has been working with amino acids dispersed in agarose gel, and with semi-conductive material, hydrogenated amorphous silicon (a-Si: H) film. In previous report, ion